

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 September 2008 (25.09.2008)

PCT

(10) International Publication Number
WO 2008/115597 A2

(51) International Patent Classification:
A61K 38/19 (2006.01)

(21) International Application Number:
PCT/US2008/003803

(22) International Filing Date: 21 March 2008 (21.03.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/919,166 21 March 2007 (21.03.2007) US

(71) Applicant (for all designated States except US): **CYTO-LOGIC, INC.** [US/US]; 2401 Research Boulevard, Suite 205, Fort Collins, CO 80526 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **HOWELL, Mark, Douglas** [US/US]; 608 Langdale Drive, Fort Collins, CO 80526 (US).

(74) Agents: **NEEDLE, William, H.** et al.; Needle & Rosenberg, P.C., Suite 1000, 999 Peachtree Street, Atlanta, GA 30309-3915 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(54) Title: CYTOKINE MUTEINS AND METHODS FOR ENHANCING IMMUNE RESPONSES IN MAMMALS

(57) Abstract: Provided are cytokine muteins and methods of use thereof for increasing an immune response in a mammal to treat various chronic diseases. Also provided is an adsorbent matrix, composed of an inert, biocompatible substrate joined covalently to one or more cytokine muteins, that is capable of specifically binding to soluble cytokine receptors.



WO 2008/115597 A2

CYTOKINE MUTEINS AND METHODS FOR ENHANCING IMMUNE RESPONSES IN MAMMALS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application number 60/919,166 filed on March 21, 2007. The aforementioned application is herein incorporated by this reference in its entirety.

BACKGROUND

[0002] This invention relates generally to the field of immunotherapy and, more specifically, to methods for enhancing host immune responses.

[0003] The immune system of mammals has evolved to protect the host against the growth and proliferation of potentially deleterious agents. These agents include infectious microorganisms such as bacteria, viruses, fungi, and parasites which exist in the environment and which, upon introduction to the body of the host, can induce varied pathological conditions. Other pathological conditions may derive from agents not acquired from the environment, but rather which arise spontaneously within the body of the host. The best examples are the numerous malignancies known to occur in mammals. Ideally, the presence of these deleterious agents in a host triggers the mobilization of the immune system to effect the destruction of the agent and, thus, restore the sanctity of the host environment.

[0004] The destruction of pathogenic agents by the immune system involves a variety of effector mechanisms which can be grouped generally into two categories: innate and specific immunity. The first line of defense is mediated by the mechanisms of innate immunity. Innate immunity does not discriminate among the myriad agents that might gain entry into the host's body. Rather, it responds in a generalized manner that employs the inflammatory response, phagocytes, and plasma-borne components such as complement and interferons. In contrast, specific immunity does discriminate among pathogenic agents. Specific immunity is mediated by B and T lymphocytes,

and it serves, in large part, to amplify and focus the effector mechanisms of innate immunity.

[0005] The elaboration of an effective immune response requires contributions from both innate and specific immune mechanisms. The function of each of these arms of the immune system individually, as well as their interaction with each other, is carefully coordinated, both in a temporal/spatial manner and in terms of the particular cell types that participate. This coordination results from the actions of a number of soluble immunostimulatory mediators or “immune system stimulators” (reviewed in, Trinchieri et al., J. Cell, Biochem. 53:301-308 (1993)). Certain of these immune system stimulators initiate and perpetuate the inflammatory response and the attendant systemic sequelae. Examples of these include, but are not limited to, the proinflammatory mediators tumor necrosis factors α and β , interleukin-1, interleukin-6, interleukin-8, interferon- γ , and the chemokines RANTES, macrophage inflammatory proteins 1- α and 1- β and macrophage chemotactic and activating factor. Other immune system stimulators facilitate interactions between B and T lymphocytes of specific immunity. Examples of these include, but are not limited to, interleukin-2, interleukin-4, interleukin-5, interleukin-6, and interferon- γ . Still other immune system stimulators mediate bidirectional communication between specific immunity and innate immunity. Examples of these include, but are not limited to, interferon- γ , interleukin-1, tumor necrosis factors and interleukin-12. All of these immune system stimulators exert their effects by binding the specific receptors on the surface of host cells, resulting in the delivery of intracellular signals that alter the function of the target cell. Cooperatively, these mediators stimulate the activation and proliferation of immune cells, recruit them to particular anatomical sites, and permit their collaboration in the elimination of the offending agent. The immune response induced in any individual is determined by the particular complement of immune system stimulators produced and by the relative abundance of each.

[0006] In contrast to the immune system stimulators described above, the immune system has evolved other soluble mediators that serve to inhibit immune responses (reviewed in Arend, Adv. Int. Med. 40:365-394 (1995)). These “immune system inhibitors” provide the immune system with the ability to dampen responses in order to prevent the establishment of a chronic inflammatory state with the potential to damage

the host's tissues. Regulation of host immune function by immune system inhibitors is accomplished through a variety of mechanisms.

[0007] A role for host-derived immune system inhibitors in chronic disease also has been established. In the majority of cases, this reflects a polarized T cell response during the initial infection, wherein the production of immunosuppressive mediators (i.e., interleukin-4, interleukin-10, and/or transforming growth factor) dominates over the production of immunostimulatory mediators (i.e., interleukin-2, interferon- γ , and/or tumor necrosis factor) (reviewed in Lucey et al., Clin. Micro. Rev. 9:532-562 (1996)). Overproduction of immunosuppressive mediators of this type has been shown to produce chronic, non-healing pathologies in a number of medically important diseases. These include, but are not limited to, diseases resulting from infection. In cases where the production of any of the aforementioned immune system inhibitors, either individually or in combination, dampens or otherwise alters immune responsiveness before the elimination of the pathogenic agent, a chronic infection may result.

[0008] In addition to this role in infectious disease, host-derived immune system inhibitors contribute also to chronic malignant disease. Compelling evidence is provided by studies of soluble tumor necrosis factor receptor Type I (sTNFRI) in cancer patients. Nanomolar concentrations of sTNFRI are synthesized by a variety of activated immune cells in cancer patients and, in many cases, by the tumors themselves (Aderka et al., Cancer Res. 51:5602-5607 (1991); Adolf and Apfler, J. Immunol. Meth. 143:127-136 (1991)). In addition, circulating sTNFRI levels often are elevated significantly in cancer patients (Aderka et al., *supra*; Kalmanti et al., Int. J. Hematol. 57:147-152 (1993); Elsasser-Beile et al., Tumor Biol. 15:17-24 (1994); Gadducci et al., Anticancer Res. 16:3125-3128 (1996); Digel et al., J. Clin. Invest. 89:1690-1693 (1992)), decline during remission and increase during advanced stages of tumor development (Aderka et al., *supra*; Kalmanti et al., *supra*; Elsasser-Beile et al., *supra*; Gadducci et al., *supra*) and, when present at high levels, correlate with poorer treatment outcomes (Aderka et al., *supra*). These observations suggest that sTNFRI aids tumor survival by inhibiting anti-tumor immune mechanisms which employ tumor necrosis factors (TNF), and they argue favorably for the clinical manipulation of sTNFRI levels as a therapeutic strategy for cancer.

[0009] That the removal of immune system inhibitors provides clinical benefit derives from the evaluation of Ultrapheresis, a promising experimental cancer therapy (Lentz, J. Biol. Response Modif. 8:511-527 (1989); Lentz, Ther. Apheresis 3:40-49 (1999); Lentz, Jpn. J. Apheresis 16:107-114 (1997)). Ultrapheresis involves extracorporeal fractionation of plasma components by ultrafiltration. Ultrapheresis selectively removes plasma components within a defined molecular size range, and it has been shown to provide significant clinical advantage to patients presenting with a variety of tumor types. Ultrapheresis induces pronounced inflammation at tumor sites, often in less than one hour post-initiation. This rapidity suggests a role for preformed chemical and/or cellular mediators in the elaboration of this inflammatory response, and it reflects the removal of naturally occurring plasma inhibitors of that response. Indeed, immune system inhibitors of TNF and interleukin-1, and interleukin-6 are removed by Ultrapheresis (Lentz, Ther. Apheresis 3:40-49 (1999)). Notably, the removal of sTNFRI has been correlated with the observed clinical responses (Lentz, Ther. Apheresis 3:40-49 (1999); Lentz, Jpn. J. Apheresis 16:107-114 (1997)).

[0010] Ultrapheresis is in direct contrast to more traditional approaches which have endeavored to boost immunity through the addition of immune system stimulators. Preeminent among these has been the infusion of supraphysiological levels of TNF (Sidhu and Bollon, Pharmacol. Ther. 57:79-128 (1993)); and of interleukin-2 (Maas et al., Cancer Immunol. Immunother. 36:141-148 (1993)), which indirectly stimulates the production of TNF. These therapies have enjoyed limited success (Sidhu and Bollon, *supra*, Maas et al., *supra*) due to the fact: 1) that at the levels employed they proved extremely toxic; and 2) that each increases the plasma levels of the immune system inhibitor, sTNFRI (Lantz et al., Cytokine 2:402-406 (1990); Miles et al., Brit. J. Cancer 66:1195-1199 (1992)). Together, these observations support the utility of Ultrapheresis as a biotherapeutic approach to cancer – one which involves the removal of immune system inhibitors, rather than the addition of immune system stimulators.

[0011] Although Ultrapheresis provides advantages over traditional therapeutic approaches, there are certain drawbacks that limit its clinical usefulness. Not only are immune system inhibitors removed by Ultrapheresis, but other plasma components, including beneficial ones, are removed since the discrimination between removed and retained plasma components is based solely on molecular size. An additional drawback

to Ultrapheresis is the significant loss of circulatory volume during treatment, which must be offset by the infusion of replacement fluid. The most effective replacement fluid is an ultrafiltrate produced, in an identical manner, from the plasma of non-tumor bearing donors. A typical treatment regimen (15 treatments, each with the removal of approximately 7 liters of ultrafiltrate) requires over 200 liters of donor plasma for the production of replacement fluid. The chronic shortage of donor plasma, combined with the risks of infection by human immunodeficiency virus, hepatitis A, B, and C or other etiologic agents, represents a severe impediment to the widespread implementation of Ultrapheresis.

SUMMARY

[0012] Provided is a method for stimulating immune responses in a mammal through the depletion of immune system inhibitors such as soluble cytokine receptors present in the circulation of the mammal. The depletion of immune system inhibitors such as soluble cytokine receptors can be effected by removing biological fluids from the mammal and contacting these biological fluids with a binding partner, for example, cytokine muteins, capable of selectively binding to the targeted immune system inhibitor(s).

[0013] Binding partners useful in these methods include cytokine muteins having specificity for soluble cytokine receptors. Moreover, mixtures of cytokine muteins having specificity for one or more soluble cytokine receptors can be used.

[0014] As an example, a binding partner, such as a cytokine mutein, can be immobilized previously on a solid support to create an "adsorbent matrix" (Figure 1). The exposure of biological fluids to such an adsorbent matrix will permit binding by the immune system inhibitor such as soluble cytokine receptor, thus, effecting a decrease in its abundance in the biological fluids. The treated biological fluid can be returned to the patient. The total volume of biological fluid to be treated and the treatment rate are parameters individualized for each patient, guided by the induction of vigorous immune responses while minimizing toxicity. The solid support (i.e., inert medium) can be composed of any material useful for such purpose including, but not limited to, for example, hollow fibers, cellulose-based fibers, synthetic fibers, flat or pleated membranes, silica-based particles, macroporous beads, and the like.

[0015] As another example, the binding partner such as a cytokine mutein can be mixed with the biological fluid in a “stirred reactor” (Figure 2). The binding partner-immune system inhibitor complex then can be removed by mechanical or by chemical or biological means or methods, and the altered biological fluid can be returned to the patient.

[0016] Also provided are conjugates comprising cytokine muteins attached to a substrate.

[0017] Further provided are apparatuses incorporating either the adsorbent matrix or the stirred reactor.

BRIEF DESCRIPTION OF THE FIGURES

[0018] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects of the invention and together with the description, serve to explain the principles of the invention.

[0019] Figure 1 schematically illustrates an “adsorbent matrix” configuration of an aspect of the disclosed compositions, conjugates and methods. In this example, blood is removed from the patient and separated into a cellular and an acellular component, or fraction thereof. The acellular component, or fraction thereof, is exposed to the adsorbent matrix to effect the binding and, thus, depletion of a targeted immune system inhibitor such as soluble cytokine receptor. The altered acellular component, or fraction thereof, then is returned contemporaneously to the patient.

[0020] Figure 2 schematically illustrates a “stirred reactor” configuration of an aspect of the disclosed compositions, conjugates and methods. In this example, blood is removed from the patient and separated into a cellular and an acellular component, or fraction thereof. A binding partner such as a cytokine mutein is added to the acellular component, or fraction thereof. Subsequently, the binding partner (cytokine mutein)/immune system inhibitor (soluble cytokine receptor) complex is removed by mechanical or by chemical or biological means or methods from the acellular component, or fraction thereof, and the altered biological fluid is returned contemporaneously to the patient.

DETAILED DESCRIPTION

[0021] The present invention may be understood more readily by reference to the following detailed description of preferred aspects of the invention and the Examples included therein and to the Figures and their previous and following description.

[0022] Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, specific nucleic acid molecules, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

[0023] As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a ribonucleic acid includes mixtures of ribonucleic acid molecules, reference to a probe includes mixtures of two or more such probes, and the like.

[0024] Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

[0025] In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

[0026] “Optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase “the sample optionally may contain more than one cytokine mutein” means that the sample may or may not contain more than one cytokine mutein and that the description includes both a sample containing one cytokine mutein and a sample containing more than one cytokine mutein.

[0027] Because of the beneficial effects associated with the removal of immune system inhibitors, there exists a need for methods which can be used to specifically deplete those inhibitors from circulation. Such methods ideally should be specific and not remove other circulatory components, and they should not result in any significant loss of circulatory volume. The disclosed methods and compositions satisfy these needs and provide related advantages as well.

[0028] Provided are methods to reduce the levels of immune system inhibitors such as soluble cytokine receptors in the circulation of a host mammal, thereby potentiating an immune response capable of resolving a pathological condition or decreasing the severity of a pathological condition. By enhancing the magnitude of the host's immune response, the disclosed methods avoid the problems associated with the repeated administration of chemotherapeutic or other agents which often have undesirable side effects, for example, chemotherapeutic or other agents used in treating cancer.

[0029] The disclosed methods generally are accomplished by: (a) obtaining a biological fluid from a mammal having a pathological condition; (b) contacting the biological fluid with a cytokine mutein binding partner capable of selectively binding to a targeted immune system inhibitor such as soluble cytokine receptor to produce an altered biological fluid having a reduced amount of the targeted immune system inhibitor; and, thereafter (c) administering the altered biological fluid to the mammal.

[0030] As used herein, the term "immune system stimulator" refers to soluble mediators that increase the magnitude of an immune response, or which encourage the development of particular immune mechanisms that are more effective in resolving a specific pathological condition. Examples of immune system stimulators include, but are not limited to, the proinflammatory mediators tumor necrosis factors, Fas ligand, CD27 ligand, CD40 ligand, interleukin-1, interleukin-2, interleukin-4, interleukin-5, interleukin-6, interleukin-8, interleukin-12, interferon- γ , and the chemokines RANTES, macrophage inflammatory proteins 1 and 1, and macrophage chemotactic and activating factor, as discussed above.

[0031] As used herein, the term "immune system inhibitor" refers to a soluble mediator that decreases the magnitude of an immune response, or which discourages the development of particular immune mechanisms that are more effective in resolving a specific pathological condition, or which encourages the development of particular

immune mechanisms that are less effective in resolving a specific pathological condition. Examples of host-derived immune system inhibitors include interleukin-1 receptor antagonist, transforming growth factor, interleukin-4, interleukin-10, or the soluble receptors for interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-7, interferon- γ , tumor necrosis factors, Fas ligand, CD27, and CD40. Immune system inhibitors produced by microorganisms are also potential targets including, for example, soluble receptors for tumor necrosis factor. As used herein, the term “targeted” immune system inhibitor refers to that inhibitor, or collection of inhibitors, which is to be removed from the biological fluid by the disclosed methods, for example, soluble cytokine receptors.

[0032] As used herein, the term “soluble cytokine receptor” refers to soluble forms of membrane receptors for interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-7, interferon- γ , tumor necrosis factors, Fas ligand, CD27, and CD40. Membrane receptors for each of these cytokines can exist also in a soluble form, and each can function as an immune system inhibitor, as discussed above.

[0033] As used herein, the term “mammal” can be a human or a non-human animal, such as dog, cat, horse, cattle, pig, sheep, non-human primate, mouse, rat, rabbit, or other mammals, for example. The term “patient” is used synonymously with the term “mammal” in describing the disclosed compositions, conjugates and methods.

[0034] As used herein, the term “pathological condition” refers to any condition where the persistence within a host of an agent, immunologically distinct from the host, is a component of or contributes to a disease state. Examples of such pathological conditions include, but are not limited to, those resulting from persistent viral, bacterial, parasitic, and fungal infections, and cancer. Among individuals exhibiting such chronic diseases, those in whom the levels of immune system inhibitors are elevated are particularly suitable for the disclosed treatment. Plasma levels of immune system inhibitors can be determined using methods well known in the art (see, for example, Adolf and Apfler, *supra*, 1991). Those skilled in the art readily can determine pathological conditions that would benefit from the depletion of immune system inhibitors according to the present methods.

[0035] As used herein, the term “biological fluid” refers to a bodily fluid obtained from a mammal, for example, blood, including whole blood, plasma, serum, lymphatic fluid,

or other types of bodily fluids. If desired, the biological fluid can be processed or fractionated, for example, to obtain an acellular component. As it relates to the disclosed compositions, conjugates and methods, the term “acellular biological fluid” refers to the acellular component of the circulatory system including plasma, serum, lymphatic fluid, or fractions thereof. The biological fluids can be removed from the mammal by any means or methods known to those skilled in the art, including, for example, conventional apheresis methods (see, Apheresis: Principles and Practice, McLeod, Price, and Drew, eds., AABB Press, Bethesda, MD (1997)). The amount of biological fluid to be extracted from a mammal at a given time will depend on a number of factors, including the age and weight of the host mammal and the volume required to achieve therapeutic benefit. As an initial guideline, one-half to four plasma volumes can be removed and, thereafter, depleted of the targeted immune system inhibitor according to the present methods.

[0036] As used herein, the term “selectively binds” means that a molecule binds to one type of target molecule, but not substantially to other types of molecules. The term “specifically binds” is used interchangeably herein with “selectively binds.”

[0037] As used herein, the term “binding partner” is intended to include any molecule chosen for its ability to selectively bind to the targeted immune system inhibitor. The binding partner can be one which naturally binds the targeted immune system inhibitor. For example, tumor necrosis factor can be used as a binding partner for sTNFRI. Alternatively, other binding partners, chosen for their ability to selectively bind to the targeted immune system inhibitor, can be used. These include fragments of the natural binding partner, polyclonal or monoclonal antibody preparations or fragments thereof, or synthetic peptides. In a further aspect, the binding partner can be a cytokine mutein which can be monomeric or multimeric.

[0038] As used herein, the term “cytokine mutein” refers to a cytokine variant having one or more amino acid substitutions or deletions relative to a parent sequence and retaining specific binding activity for a cytokine receptor, either soluble and/or membrane bound.

[0039] Provided are compositions and methods for stimulating or enhancing an immune response in a mammal. The invention advantageously uses ligands that bind to immune system inhibitors to counterbalance or decrease the dampening effect of immune system

inhibitors on the immune response. Such ligands, also referred to herein as “binding partners,” can be attached to a solid support to allow the removal of an immune system inhibitor from a biological fluid.

[0040] A binding partner particularly useful in the present invention is a ligand that binds with high affinity to an immune system inhibitor, for example, a soluble cytokine receptor. Another useful characteristic of a binding partner is a lack of direct toxicity. For example, a binding partner lacking or having reduced cytokine agonist activity is particularly useful. Generally, even when a ligand such as a binding partner is covalently bound to a solid support, a certain percentage of the bound ligand will leach from the support, for example, via chemical reactions that break down the covalent linkage or protease activity present in a biological fluid. In such a case, the ligand will leach into the biological fluid being processed and, thus, be returned to the patient. Therefore, it is advantageous to use a ligand that has affinity for an immune system inhibitor but has decreased ability to stimulate a biological response, that is, has decreased or low agonist activity. In this case, even if some of the ligand leaches into the processed biological fluid, the ligand would still exhibit low biological activity with respect to membrane receptor signaling when reintroduced into the patient.

[0041] Yet another useful characteristic of a binding partner is a lack of indirect toxicity, for example, immunogenicity. As discussed above, it is common for a bound ligand to leach from a matrix, resulting in the ligand being present in the processed biological fluid. When the biological fluid is returned to the patient, this results in the introduction of a low level of the ligand to the patient. If the ligand is immunogenic, an immune response against the ligand can be stimulated, resulting in undesirable immune responses, particularly in a patient in which the process is being repeated. Therefore, a ligand having low immunogenicity would minimize any undesirable immune responses against the ligand. As disclosed herein, a particularly useful ligand to be used as a binding partner of the invention is derived from the same species as the patient being treated. For example, for treating a human, a human cytokine mutein can be used as the binding partner, which is expected to have low immunogenicity given its homology to the endogenous cytokine. Similarly, muteins derived from other mammalian species can be used in the respective species.

[0042] One advantage to using cytokine muteins as binding partners in the present invention is that cytokine muteins can exhibit decreased signaling through membrane receptors, for example, decreased cytotoxic activity or *in vivo* toxicity, relative to the wild-type cytokines, while retaining the ability to bind soluble cytokine receptors. As discussed above, such a reduced signaling through membrane receptors, for example, reduced cytotoxicity or *in vivo* toxicity, is advantageous in view of the potential leaching of the ligand from a matrix and introduction of low levels into a patient when an altered biological fluid is returned to the patient.

[0043] An additional advantage of using cytokine muteins is that they can adopt a wild-type structure. Because the muteins are highly homologous to the wild-type cytokine sequence, these muteins can fold into a wild-type structure that retains cytokine receptor binding activity. Such a wild-type structure means that the same amino acid residues are exposed on the surface of the molecule as in the wild-type cytokine, except for possibly the mutant amino acid residue. Such a wild-type folding means that the cytokine muteins should have little or no immunogenicity in the respective mammalian species.

[0044] As disclosed herein, particularly useful muteins are muteins of human interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-7, interferon- γ , tumor necrosis factors, Fas ligand, CD27, CD40, and the analogous muteins in other mammalian species. Specific exemplary muteins, which exhibit decreased signaling through their respective membrane receptors while retaining the majority of the binding activity of the wild type parent cytokine, include the following: a mutein of human IL-1 β (SEQ ID NO:1), wherein Thr⁹ is substituted with Gly (SEQ ID NO:2) (Camacho, N.P., et al., Biochem. 32:8749-8757 (1993)); a mutein of human IL-1 β wherein Arg¹²⁷ (Arg¹¹ in the sequence of the processed, mature polypeptide) is substituted with Gly (SEQ ID NO:3) (Gehrke, L., et al., J. Biol. Chem. 11:5922-5925 (1990)); a mutein of human IL-2 (SEQ ID NO:4), wherein Asp²⁰ is substituted with Asn (SEQ ID NO:5) (Weigel, U., et al. Eur. J. Biochem. 180:295-300 (1989)); and, a mutein of human IL-2 wherein Cys¹²⁵ is deleted (SEQ ID NO:6) (Liang, S.-M., et al. J. Biol. Chem. 263:4768-4772 (1988)). Included also are muteins of human IL-4 (SEQ ID NO:7) that contain amino acid substitutions in helix D, the receptor activating region of the cytokine. Specific mutations include the substitution of Asp for R¹²¹ (SEQ ID NO:8), Asp for

Y¹²⁴ (SEQ ID NO:9) and Asp for Ser¹²⁵ (SEQ ID NO:10) (Tony, H.P., et al., Eur. J. Biochem. 225:659-665 (1994). Further, the substitution of Asp for both R¹²¹ and Y¹²⁴ (SEQ ID NO:11) has been shown to dissociate binding and receptor activation most efficiently (Tony, H.P., et al., *supra*, 1994).

[0045] It is understood that cytokine muteins additional to the specific muteins exemplified herein can be used in methods of the invention.

[0046] One skilled in the art can readily determine additional muteins suitable for use in the disclosed compositions, conjugates and methods. As discussed above, cytokine muteins having relatively high affinity for cytokine receptors and decreased signaling through membrane receptors, for example, decreased cytotoxicity or *in vivo* toxicity, relative to wild-type cytokines are particularly useful in the disclosed compositions, conjugates and methods. One skilled in the art can readily determine additional suitable cytokine muteins based on methods well known to those skilled in the art. Methods for introducing amino acid substitutions into a sequence are well known to those skilled in the art (Ausubel et al., Current Protocols in Molecular Biology (Supplement 56), John Wiley & Sons, New York (2001); Sambrook and Russel, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor (2001); U.S. Patent Nos. 5,264,563 and 5,523,388). Generation of cytokine muteins has been previously described (Van Ostade et al., *supra*, 1994; Van Ostade et al., *supra*, 1991; Zhang et al., *supra*, 1992; Yamagishi et al., *supra*, 1990; Gehrke, L., et al., *supra*, 1990; Camacho, N.P., et al., *supra*, 1993; Liang, S.-M., et al. *supra*, 1988; Weigel, U., et al. *supra*, 1989; Tony, H.P., et al., *supra*, 1994; and, Grunewald, S. M., et al. *supra*, 1997). Furthermore, one skilled in the art can readily determine the binding and cytotoxicity and/or *in vivo* toxicity of candidate muteins to ascertain the suitability for use in the disclosed method (Van Ostade et al., *supra*, 1994; Van Ostade et al., *supra*, 1991; Zhang et al., *supra*, 1992; Yamagishi et al., *supra*, 1990).

[0047] Provided are a variety of cytokine muteins as disclosed herein. Generally, a particularly useful cytokine mutein has about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, about 10-fold, about 11-fold, about 12-fold, about 13-fold, about 14-fold, about 15-fold, about 16-fold, about 17-fold, about 18-fold, about 19-fold, about 20-fold, about 25-fold, about 30-fold, or even higher fold reduced binding affinity for cytokine receptors, particularly membrane

bound cytokine receptors, relative to the wild type cytokine. Such reduced binding affinity can be, but is not necessarily, exhibited toward soluble cytokine receptors. Also, a particularly useful cytokine mutein has about 5-fold, about 10-fold, about 50-fold, about 100-fold, about 150-fold, about 200-fold, about 300-fold, about 500-fold, about 1000-fold, about 2000-fold, about 3000-fold, about 4000-fold, about 5000-fold, about 6000-fold, about 7000-fold, about 8000-fold, about 9000-fold, about 10,000-fold, about 20,000-fold, about 30,000-fold, about 50,000-fold, or even higher fold reduced signaling through the membrane receptors, for example, reduced cytotoxicity or *in vivo* toxicity, relative to the wild type cytokine. It is understood that a cytokine mutein can have reduced binding affinity and/or reduced cytotoxicity, as discussed above and disclosed herein.

[0048] Provided is a conjugate comprising a cytokine mutein attached to a substrate.

[0049] Further provided is a method of stimulating an immune response in a mammal having a pathological condition. The method can include the steps of obtaining a biological fluid from the mammal; contacting the biological fluid with a cytokine mutein having specific binding activity for a soluble cytokine receptor; removing the cytokine mutein bound to the soluble cytokine receptor from the biological fluid to produce an altered biological fluid having a reduced amount of soluble cytokine receptor; and administering the altered biological fluid to the mammal. The biological fluid can be, for example, blood, plasma, serum or lymphatic fluid, including whole blood. In a further aspect, a method using whole blood as the biological fluid can further include the step of separating the whole blood into a cellular component and an acellular component or a fraction of the acellular component, wherein the acellular or the fraction of the acellular component contains a soluble cytokine receptor. The method can additionally include the step of combining the cellular component with the altered acellular component or altered fraction of the acellular component to produce altered whole blood, which can be administered to the mammal as the altered biological fluid. Accordingly, the cellular component and the altered acellular component or altered fraction of the acellular component can be administered separately to the mammal.

[0050] Various mixtures of binding partners can be used. For example, one mixture can be composed of multiple binding partners that selectively bind to a single targeted

immune system inhibitor. Another mixture can be composed of multiple binding partners, each of which selectively binds to different targeted immune system inhibitors. Alternatively, the mixture can be composed of multiple binding partners that selectively bind to different targeted immune system inhibitors.

[0051] When it is desirable to increase the molecular weight of the binding partner/immune system inhibitor complex, the binding partner can be conjugated to a carrier. Examples of such carriers include, but are not limited to, proteins, complex carbohydrates, and synthetic polymers such as polyethylene glycol.

[0052] As used herein, “functionally active binding sites” of a binding partner refer to sites that are capable of binding to one or more targeted immune system inhibitors.

[0053] Methods for producing the various binding partners useful with the disclosed compositions, conjugates and methods are well known to those skilled in the art. Such methods include, for example, recombinant DNA and synthetic techniques, or a combination thereof. Binding partners such as cytokine muteins can be expressed in prokaryotic or eukaryotic cells, for example, mammalian, insect, yeast, and the like. If desired, codons can be changed to reflect any codon bias in a host species used for expression.

[0054] A binding partner, such as a cytokine mutein, can be attached to an inert medium to form an adsorbent matrix (Figure 1). The cytokine mutein can be, for example, covalently attached to a substrate such as an inert medium. As used herein, the term “inert medium” is intended to include solid supports to which the binding partner(s) can be attached. Particularly useful supports are materials that are used for such purposes including, for example, cellulose-based hollow fibers, synthetic hollow fibers, silica-based particles, flat or pleated membranes, macroporous beads, agarose-based particles, and the like. The inert medium can be in the form of a bead, for example, a macroporous bead or a non-porous bead. Exemplary macroporous beads include, but are not limited to, naturally occurring materials such as agarose, cellulose, controlled pore glass, or synthetic materials such as polyacrylamide, crosslinked agarose (such as Trisacryl[™], Sephacryl, Actigel[™], and Ultrogel[™]), azlactone, polymethacrylate, polystyrene/divinylbenzene, and the like. In one aspect, a macroporous bead comprises Actigel[™]. Exemplary non-porous beads include, but are not limited to, silica, polystyrene, latex, and the like. Hollow fibers and membranes can

also be composed of natural or synthetic materials. Exemplary natural materials include, but are not limited to, cellulose and modified cellulose, for example, cellulose diacetate or triacetate. Exemplary synthetic materials include, but are not limited to, polysulfone, polyvinyl, polyacetate, and the like. Such inert media can be obtained commercially or can be readily made by those skilled in the art. The binding partner can be attached to the inert medium by any means or methods known to those skilled in the art including, for example, covalent conjugation. Alternatively, the binding partner can be associated with the inert matrix through high-affinity, non-covalent interaction with an additional molecule which has been covalently attached to the inert medium. For example, a biotinylated binding partner can interact with avidin or streptavidin previously conjugated to the inert medium.

[0055] The adsorbent matrix thus produced can be contacted with a biological fluid, or a fraction thereof, through the use of an extracorporeal circuit. The development and use of extracorporeal, adsorbent matrices has been extensively reviewed (see Kessler, Blood Purification 11:150-157 (1993)).

[0056] In a further aspect, herein referred to as the “stirred reactor” (Figure 2), the biological fluid can be exposed to the binding partner such as a cytokine mutein in a mixing chamber and, thereafter, the binding partner/immune system inhibitor complex can be removed by means or methods known to those skilled in the art, including, for example, by mechanical or by chemical or biological separation methods. For example, a mechanical separation method can be used in cases where the binding partner, and therefore the binding partner/immune system inhibitor complex, represent the largest components of the treated biological fluid. In those cases, filtration can be used to retain the binding partner and immune system inhibitors associated therewith, while allowing all other components of the biological fluid to permeate through the filter and, thus, to be returned to the patient. In an example of a chemical or biological separation method, the binding partner and immune system inhibitors associated therewith can be removed from the treated biological fluid through exposure to an adsorbent matrix capable of specifically attaching to the binding partner. For example, a matrix constructed with antibodies reactive with a cytokine mutein can serve this purpose. Similarly, were biotin conjugated to the binding partner such as a cytokine mutein prior to its addition to the biological fluid, a matrix constructed with avidin or streptavidin

could be used to deplete the binding partner and immune system inhibitors associated therewith from the treated fluid. It is understood that removal of the binding partner/immune system inhibitor complex, such as cytokine mutein bound to soluble cytokine receptor, from a biological fluid can be accomplished by separating the biological fluid and the binding partner/immune system inhibitor complex in any suitable manner. Either or both the binding partner/immune system inhibitor complex and the biological fluid can be passively or actively separated from the other. Thus, for example, removal of cytokine mutein bound to soluble cytokine receptor from a biological fluid can be accomplished by, for example, actively removing cytokine mutein bound to soluble cytokine receptor from the biological fluid or actively removing the biological fluid from the cytokine mutein bound to soluble cytokine receptor.

[0057] In a final step of the present methods, the treated or altered biological fluid, having a reduced amount of targeted immune system inhibitor such as soluble cytokine receptor, can be returned to the patient receiving treatment along with untreated fractions of the biological fluid, if any such fractions were produced during the treatment. The altered biological fluid can be administered to the mammal by any means or methods known to those skilled in the art, including, for example, by infusion directly into the circulatory system. The altered biological fluid can be administered immediately after contact with the binding partner in a contemporaneous, extracorporeal circuit. In this circuit, the biological fluid can be (a) collected, (b) separated into cellular and acellular components, if desired, (c) exposed to the binding partner, and if needed, separated from the binding partner bound to the targeted immune system inhibitor, (d) combined with the cellular component, if needed, and (e) readministered to the patient as altered biological fluid. In a further aspect, the altered acellular biological fluid can be administered to the patient at an infusion site different from the site where the cellular component of the biological fluid is administered to the patient. The administration of the altered acellular biological fluid to the patient can be simultaneous with, precede, or follow the administration of the cellular component of the biological fluid to the patient. Alternatively, the administration of the altered biological fluid can be delayed under appropriate storage conditions readily determined by those skilled in the art.

[0058] If desirable, the entire process can be repeated. Those skilled in the art can readily determine the benefits of repeated treatment by monitoring the clinical status of the patient, and correlating that status with the concentration(s) of the targeted immune system inhibitor(s) such as soluble cytokine receptor in circulation prior to, during, and after treatment.

[0059] Further provided is an apparatus for reducing the amount of a targeted immune system inhibitor such as soluble cytokine receptor in a biological fluid. The apparatus can be composed of: (a) a means for separating the biological fluid into a cellular component and an acellular component or fraction thereof; (b) an adsorbent matrix having attached thereto a cytokine mutein or a stirred reactor as described above to produce an altered acellular component or fraction thereof; and (c) a means for combining the cellular fraction with the altered acellular component or fraction thereof. The apparatus is particularly useful for whole blood as the biological fluid in which the cellular component is separated either from whole plasma or a fraction thereof.

[0060] The means for initially fractionating the biological fluid into the cellular component and the acellular component, or a fraction thereof, and for recombining the cellular component with the acellular component, or fraction thereof, after treatment are known to those skilled in the art (see Apheresis: Principles and Practice, *supra*).

[0061] Immune system inhibitors to be targeted can be soluble forms of membrane receptors for interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-7, interferon- γ , tumor necrosis factors, Fas ligand, CD27, and CD40.

[0062] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in C or is at ambient temperature, and pressure is at or near atmospheric.

EXAMPLE 1

Production, Purification, and Characterization of Immune System Inhibitors

[0063] Immune system inhibitors useful in perfecting and practicing the invention can be produced recombinantly either in *E. coli* or other bacterial genera, or in eukaryotic cell culture essentially as described (see U.S. Patent No. 6,379,708, which is incorporated herein by reference). The construction of expression plasmids, the methods for transforming and selecting cultured cells, for purifying the immune system inhibitors, and associated assay methods (e.g., ELISA, immunoblot, SDS-PAGE) are well known in the art.

EXAMPLE 2

Production, Purification, and Characterization of Cytokine Muteins

[0064] Cytokine muteins also can be produced recombinantly either in *E. coli* or other bacterial genera, or in eukaryotic cell culture essentially as described (see U.S. Patent Application No. 11/234,057, which is incorporated herein by reference). The construction of expression plasmids, the methods for transforming and selecting cultured cells, for purifying the cytokine muteins, and associated assay methods (e.g., ELISA, immunoblot, SDS-PAGE) are well known in the art.

EXAMPLE 3

Production and *in vitro* Characterization of Cytokine Mutein Adsorbent Matrices

[0065] Cytokine muteins, produced as described above, can be immobilized on a solid support to create adsorbent matrices. For example, purified cytokine muteins can be covalently conjugated to macroporous beads such as cyanogen bromide (CNBr) Sepharose™ 4B, Actigel ALD, and others, according to the manufacturer's instructions. The resulting matrices can be packed in individual column housings and washed extensively with phosphate buffered saline prior to use.

[0066] Depletion of immune system inhibitors from normal human plasma using a cytokine mutein adsorbent matrix initially can be evaluated *in vitro*. For example, plasma may be spiked with known concentrations of purified immune system inhibitors, passed through the adsorbent matrix, and the effluent collected. Capture ELISA can be

used to quantify the levels of the immune system inhibitor in the pre- and post-matrix plasma samples. The efficiency of immune system inhibitor depletion, thus, can be calculated.

EXAMPLE 4

***Ex Vivo* Depletion of Immune System Inhibitors Using an Extracorporeal Adsorbent Device Constructed with Cytokine Muteins**

[0067] The ability of a cytokine mutein adsorbent device to deplete immune system inhibitors from plasma, when used in an extracorporeal circuit not unlike that employed for therapeutic plasma exchange, is readily evaluated. Briefly, blood is removed from a mammal and delivered to a Cobe Spectra, a centrifugal plasma separator, using a Cobe Spectra Therapeutic Plasma Exchange disposable tubing set. Once separated, the blood cells and plasma are routed, independent of each other, through the remainder of the system. The plasma component is passed through the cytokine mutein adsorbent device as illustrated in Figure 1 herein. The treated plasma is recombined with the blood cells and returned through the catheter to the mammal. One-half to four plasma volumes can be treated during a single session, and the treatment may be repeated.

[0068] Depletion of targeted immune system inhibitors by this process also is evaluated readily. Plasma samples can be obtained from the extracorporeal circuit immediately prior to entering the adsorbent device and immediately after exiting the adsorbent device. The levels of the targeted immune system inhibitor(s) in these plasma samples can be determined using capture ELISA and the efficiency of depletion can be calculated.

[0069] Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this pertains.

[0070] Although the invention has been described with reference to the presently preferred aspects, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. A conjugate comprising a cytokine mutein attached to a substrate.
2. The conjugate of claim 1, wherein the cytokine mutein is from a species selected from the group consisting of human, dog, cat, horse, sheep, goat, pig, cow, rabbit and rat.
3. The conjugate of claim 1, wherein the cytokine mutein is covalently attached to the substrate.
4. The conjugate of claim 1, wherein the substrate is an inert medium.
5. The conjugate of claim 4, wherein the inert medium is a hollow fiber.
6. The conjugate of claim 4, wherein the inert medium is in the form of a bead.
7. The conjugate of claim 6, wherein the bead is a macroporous bead.
8. The conjugate of claim 7, wherein the macroporous bead comprises a material selected from the group consisting of agarose, cross-linked agarose, cellulose, controlled pore glass, polyacrylamide, azlactone, polymethacrylate, and polystyrene.
9. The conjugate of claim 6, wherein the bead is a non-porous bead.
10. The conjugate of claim 9, wherein the non-porous bead comprises a material selected from the group consisting of silica, polystyrene and latex.
11. The conjugate of claim 4, wherein the inert medium is a cellulose-based fiber.
- 12.. The conjugate of claim 4, wherein the inert medium is a synthetic fiber.
13. The conjugate of claim 4, wherein the inert medium is a flat or pleated membrane.
14. The conjugate of claim 4, wherein the inert medium is a silica-based particle.
15. The conjugate of claim 4, where the inert medium is an agarose-based particle.
16. The conjugate of claim 1, wherein the cytokine mutein is multimeric.
17. The conjugate of claim 16, wherein the multimeric cytokine mutein comprises two or more identical amino acid sequences.

18. The conjugate of claim 16 wherein the multimeric cytokine mutein comprises two or more non-identical amino acid sequences.
19. The conjugate of claim 1, wherein the cytokine mutein is monomeric.
20. The conjugate of claim 1, wherein the cytokine mutein has reduced agonist activity relative to a wild-type cytokine.
21. The conjugate of claim 1, wherein the cytokine mutein has decreased signaling through membrane receptors relative to a wild-type cytokine.
22. The conjugate of claim 1, wherein the cytokine mutein has decreased cytotoxic activity relative to a wild-type cytokine.
23. The conjugate of claim 1, wherein the cytokine mutein has decreased *in vivo* toxicity relative to a wild-type cytokine.
24. A method of stimulating an immune response in a mammal having a pathological condition, comprising:
 - (a) obtaining a biological fluid from the mammal;
 - (b) contacting the biological fluid with a cytokine mutein having specific binding activity for a soluble cytokine receptor;
 - (c) removing the cytokine mutein bound to the soluble cytokine receptor from the biological fluid to produce an altered biological fluid having a reduced amount of soluble cytokine receptor; and
 - (d) administering the altered biological fluid to the mammal.
25. The method of claim 24, wherein the cytokine mutein is from the same species as the mammal.
26. The method of claim 24, wherein the cytokine mutein is from a species different from the species of the mammal.
27. The method of claim 24, wherein the biological fluid is selected from the group consisting of blood, plasma, serum and lymphatic fluid.

28. The method of claim 27, wherein the blood is whole blood.
29. The method of claim 28, further comprising the step of separating the whole blood into a cellular component and an acellular component or a fraction of the acellular component, wherein the acellular or the fraction of the acellular component contains a soluble cytokine receptor.
30. The method of claim 29, further comprising the step of combining the cellular component with the altered acellular component or altered fraction of the acellular component to produce altered whole blood.
31. The method of claim 24, wherein the cytokine mutein has specific binding activity for a single immune system inhibitor.
32. The method of claim 31, wherein the immune system inhibitor is a soluble cytokine receptor.
33. The method of claim 24, wherein the biological fluid is contacted with a cytokine mutein having specific binding activity for more than one immune system inhibitor.
34. The method of claim 24, wherein the cytokine mutein is attached to an inert medium to form an adsorbent matrix.
35. The method of claim 34, wherein the cytokine mutein is covalently attached to the inert medium.
36. The method of claim 34, wherein the inert medium is a hollow fiber.
37. The method of claim 34, wherein the inert medium is a macroporous bead.
38. The method of claim 34, wherein the inert medium is a cellulose-based fiber.
39. The method of claim 34, wherein the inert medium is a synthetic fiber.
40. The method of claim 34, wherein the inert medium is a flat or pleated membrane.
41. The method of claim 34, wherein the inert medium is a silica-based particle.
42. The method of claim 24, wherein the cytokine mutein is produced recombinantly.
43. The method of claim 24, wherein the biological fluid is contacted with a plurality of cytokine muteins.

44. The method of claim 43, wherein the plurality of cytokine muteins has specific binding activity for a single type of immune system inhibitor.
45. The method of claim 44, wherein the immune system inhibitor is a soluble cytokine receptor.
46. The method of claim 24, wherein the biological fluid is contacted with a plurality of cytokine muteins having specific binding activity for more than one type of immune system inhibitor.
47. The method of claim 46, wherein the plurality of cytokine muteins is attached to a carrier.
48. The method of claim 24, wherein steps (a) through (d) are repeated.
49. The method of claim 24, wherein the mammal is human.
50. The method of claim 24, wherein the mammal is non-human.
51. The method of claim 24, wherein the cytokine mutein bound to the immune system inhibitor is removed by mechanical methods.
52. The method of claim 24, wherein the cytokine mutein bound to the immune system inhibitor is removed by chemical or biological methods.
53. The method of claim 24, wherein the cytokine mutein bound to the immune system inhibitor is removed by separating the biological fluid from the cytokine mutein.
54. A method for stimulating an immune system response in a mammal having a pathological condition, comprising:
- (a) obtaining a biological fluid from the mammal;
 - (b) contacting the biological fluid with at least one cytokine mutein having specific binding activity for an immune system inhibitor, wherein the cytokine mutein is attached to an inert medium to form an adsorbent matrix;
 - (c) removing the adsorbent matrix comprising the cytokine mutein bound to the immune system inhibitor from the biological fluid to produce an altered biological fluid;
- and

(d) administering the altered biological fluid to the mammal.

55. The method of claim 54, wherein the biological fluid is selected from the group consisting of blood, plasma, serum and lymphatic fluid.

56. The method of claim 55, wherein the blood is whole blood.

57. The method of claim 56, further comprising the step of separating the whole blood into a cellular component and an acellular component or a fraction of the acellular component, wherein the acellular or the fraction of the acellular component contains an immune system inhibitor.

58. The method of claim 57, further comprising the step of combining the cellular component with the altered acellular component or altered fraction of the acellular component to produce altered whole blood.

59. A method for stimulating an immune response in a mammal having a pathological condition comprising:

(a) obtaining a biological fluid from the mammal;

(b) contacting the biological fluid with two or more cytokine muteins having specific binding activity for at least one immune system inhibitor;

(c) isolating the cytokine muteins bound to the immune system inhibitor from the biological fluid to produce an altered biological fluid; and

(d) administering the altered biological fluid to the mammal.

60. The method of claim 59, wherein the two or more cytokine muteins are attached to an inert medium to form an adsorbent matrix.

61. The method of claim 60, wherein the two or more cytokine muteins are covalently joined to the inert medium.

62. The method of claim 59, wherein the biological fluid is selected from the group consisting of blood, plasma, serum and lymphatic fluid.

63. The method of claim 62, wherein the blood is whole blood.

64. The method of claim 63, further comprising the step of separating the whole blood into a cellular component and an acellular component or a fraction of the acellular component, wherein the acellular or the fraction of the acellular component contains an immune system inhibitor.

65. The method of claim 64, further comprising the step of combining the cellular component with the altered acellular component or altered fraction of the acellular component to produce altered whole blood.

1/2

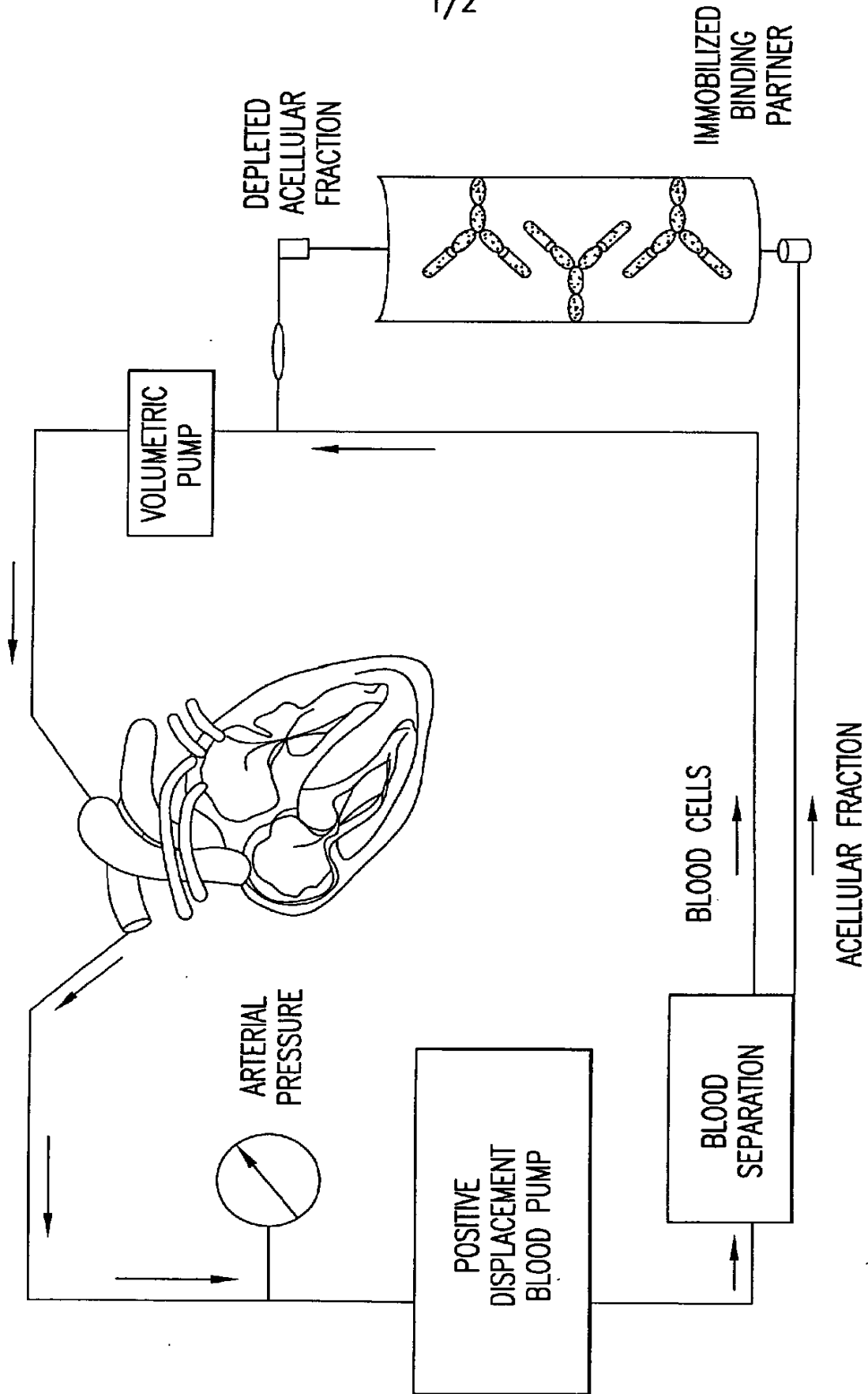


FIG. 1

2/2

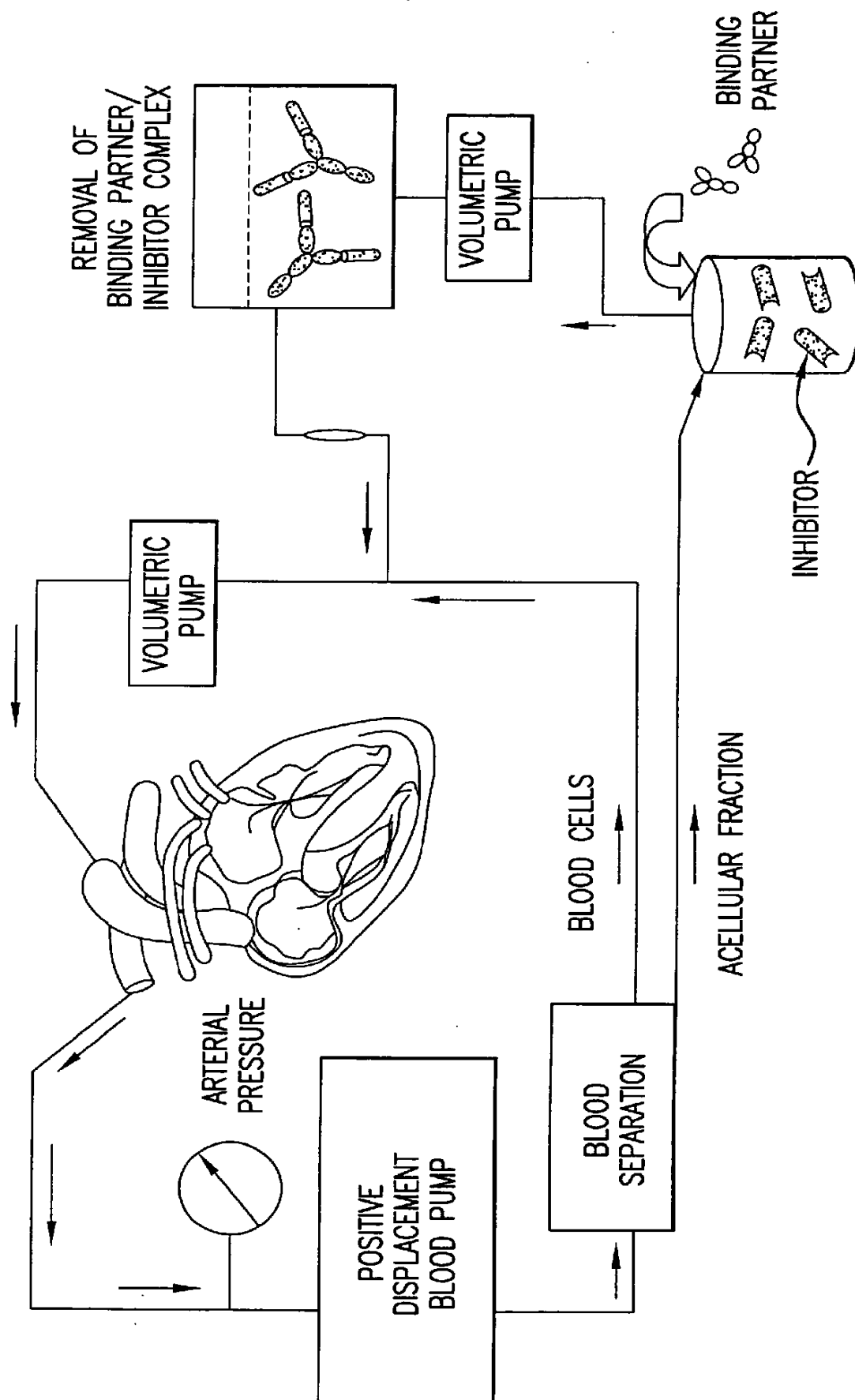


FIG.2